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(54) Title: THE USE OF A COMPOUND WITH A NEGATIVELY CHARGED DOMAIN OF RADICALS FOR THE TREATMENT OF RESTENOSIS

(57) Abstract: There is disclosed the use of a compound containing a high density negatively charged domain of vicinally oriented radicals for the preparing of a medicament for preventing, alleviating or combating restenosis in mammals including man.

THE USE OF A COMPOUND WITH A NEGATIVELY CHARGED DOMAIN OF RADICALS FOR THE TREATMENT OF RESTENOSIS

The present invention relates to the use of a compound containing a high density, negatively charged domain of vicinally oriented radicals for the preparing of a medicament for preventing, alleviating or combatting restenosis in mammals including man.

Procedures involving the use of balloon dilatation catheters, stents and other percutaneously delivered interventional devices are commonly performed on patients with coronary heart disease (CHD). The balloon angioplasty is the strategy of choice in the majority of cases and the procedure is often followed by stenting. Despite the fact that centres undertaking such procedures are properly equipped and staffed, restenosis, i.e. reocclusion of the vessels, is occurring in 30-50% of the patients treated.

In 1995 nearly 300.000 percutaneous coronary interventions were carried out in Europe and the corresponding figure for the US was approximately 500.000 and these figures continue to increase world wide. Another 500.000 coronary artery bypass procedures were performed in the US. The average cost is more than 20.000 USD per procedure. The costs for the health care system to repeat angioplasty procedures to treat restenosis is estimated to exceed USD 2,5 billion annually.

Restenosis is considered to be one of the major limitations of percutanous transluminal coronary angioplasty. Risk factors for the development of restenosis are multifactoral and mostly unknown. The traditional atherosclerotic risk factors such as hypertension, smoking and cholesterol have not been associated with restenosis. The mechanism for the process of restenosis involves many factors including vasoconstriction, migration and proliferation of smooth muscle cells, the release of regulatory substances such

as growth factors, synthesis of extracellular matrices, neointimal formation, and remodelling of vessels.

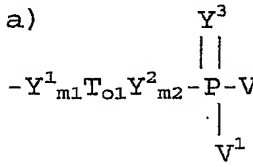
In an effort to halt restenosis, more than 50 drugs have been investigated but none have shown to be efficient. As a commonly used compound, heparin is administered during the procedures in order to counteract complications related to increased coagulation but evidence are accumulating towards the direction that heparin could promote the process of restenosis.

According to the present invention it has surprisingly become possible to use a compound containing a high density, negatively charged domain of vicinally oriented radicals for the preparing of a medicament for preventing, alleviating or combatting restenosis in mammals including man. Furthermore the invention describes the use of a compound containing a high density, negatively charged domain of vicinally oriented radicals for the preparing of a medicament for preventing, alleviating or combatting hyperplasia, remodelling and hypertrophy in mammals including man.

In preferred embodiments of the invention the negatively charged domain comprises at least three vicinal phosporus-containing radicals.

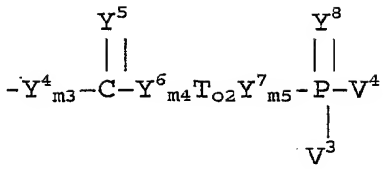
The invention also relates to the use of a compound wherein the phosphorus-containing radicals have the following formula:

a)



or

b)



wherein

V^1 to V^4 are $Y^8{}_{m6}T_{o3}U$

T_{o1} to T_{o3} are $(CH_2)_n$, CHCH, or $CH_2CHCHCH_2$

o1 to o3 are 0 to 1

n is 0 to 4

U is $R^1Y^9{}_{m7}$, $CY^{10}Y^{11}R^2$, $SY^{12}Y^{13}Y^{14}R^3$, $PY^{15}Y^{16}Y^{17}R^4R^5$, $Y^{18}PY^{19}Y^{20}Y^{21}R^6R^7$, CH_2NO_2, $NHSO_2R^8$ or $NHCY^{22}Y^{23}R^9$

m1 to m7 are 0 to 1

Y^1 to Y^{23} are N R^{10}, NOR^{11}, O or S

and where R^1 to R^{11} are

i) hydrogen

ii) a straight or branched saturated or unsaturated alkyl residue containing 1-22 carbon atoms

iii) a saturated or unsaturated aromatic or non-aromatic homo- or heterocyclic residue containing 3-22 carbon atoms and 0-5 heteroatoms consisting of nitrogen, oxygen or sulfur

iv) a straight or branched saturated or unsaturated alkyl residue containing 1-22 carbon atoms substituted with a saturated or unsaturated aromatic or non-aromatic homo- or heterocyclic residue containing 3-22 carbon atoms and 0-5 heteroatoms consisting of nitrogen, oxygen or sulfur

v) an aromatic or non-aromatic homo- or heterocyclic residue containing 3-22 carbon atoms and 0-5 heteroatoms consisting of nitrogen, oxygen or sulfur substituted with a straight or branched saturated or

unsaturated alkyl residue containing 1-22 carbon atoms.

in the said groups ii-v the residues and/or the substituents thereof being substituted with 0-6 of the following groups: hydroxy, alkoxy, aryloxy, acyloxy, carboxy, alkoxycarbonyl, alkoxycarbonyloxy, aryloxycarbonyl, aryloxycarbonyloxy, carbamoyl, fluoro, chloro, bromo, azido, cyano, oxo, oxa, amino, imino, alkylamino, arylamino, acylamino, arylazo, nitro, alkylthio or alkylsulfonyl.

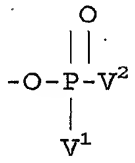
The straight or branched saturated or unsaturated alkyl residue in groups i-v above can be exemplified by methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, heneicosyl, doeicosyl, isopropyl, isobutyl, isopentyl, isohexyl, isoheptyl, isooctyl, isononyl, isodecyl, isodoecosyl, 2-butyl, 2-pentyl, 2-hexyl, 2-heptyl, 2-octyl, 2-nonyl, 2-decyl, 2-doeicosyl, 2- methylbutyl, 2-methylpentyl, 2-methylhexyl, 2-methylheptyl, 2-methyloctyl, 2-methylnonyl, 2- methyldecyl, 2-methyleicosyl, 2-ethylbutyl, 2-ethylpentyl, 2-ethylhexyl, 2-ethylheptyl, 2-ethyloctyl, 2-ethylnonyl, 2-ethyldecyl, 2-ethyleicosyl, tertbutyl, ethenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl, undecenyl, dodecenyl, tridecenyl, tetradecenyl, pentadecenyl, hexadecenyl, heptadecenyl, octadecenyl, nonadecenyl, eicosenyl, heneicosenyl, doeicosenyl, butadienyl, pentadienyl, hexadienyl, heptadienyl, octadienyl, nonadienyl, decadienyl, doeicodienyl, ethynyl, propynyl, doeicosynyl.

The saturated or unsaturated aromatic or non-aromatic homo- or heterocyclic residue in groups i-v above can be exemplified by cyclopropyl, cyclobutyl, cyclopentyl,

cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl, cyclododecyl, cyclotridecyl, cyclotetradecyl, cyclopentadecyl, cyclohexadecyl, cycloheptadecyl, cyclooctadecyl, cyclononadecyl, cycloeicosyl, cycloheneicosyl, cyclodoeicosyl, adamantyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, cyclononenyl, cyclodecenyl, phenyl, biphenyl, naphthyl, hydroxyphenyl, aminophenyl, mercaptophenyl, fluorophenyl, chlorophenyl, azidophenyl, cyanophenyl, carboxyphenyl, alkoxyphenyl, acyloxyphenyl, acylphenyl, oxiranyl, thiiranyl, aziridinyl, oxetanyl, thietanyl, azetidinyl, tetrahydrofuranyl, tetrahydrothiophenyl, pyrrolidinyl, tetrahydropyranyl, tetrahydrothiopyranyl, piperidinyl, quinuclidinyl, dioxanyl, dithianyl, trioxanyl, furyl, pyrrolyl, thienyl, pyridyl, quinolyl, benzofuryl, indolyl, benzothienyl, oxazolyl, imidazolyl, thiazolyl, pyridazinyl, pyrimidyl, pyrazinyl, purinyl or a carbohydrate.

Substituents may be selected from the group of: hydroxy, alkoxy, aryloxy, acyloxy, carboxy, alkoxycarbonyl, alkoxycarbonyloxy, aryloxycarbonyl, aryloxycarbonyloxy, carbamoyl, fluoro, chloro, bromo, azido, cyano, oxo, oxa, amino, imino, alkylamino, arylamino, acylamino, nitro, alkylthio, alkylsulfonyl.

Furthermore the invention relates to the use wherein the phosphorus-containing radicals have the following formula:



wherein V^1 and V^2 are OH, $(CH_2)_p$ OH, COOH, $CONH_2$, CONOH, $(CH_2)_pCOOH$, $(CH_2)_pCONH_2$, $(CH_2)_pCONOH$, $(CH_2)_pSO_3$ H, $(CH_2)_pSO_3$, NH_2, $(CH_2)_pNO_2$, $(CH_2)_pPO_3H_2$, $O(CH_2)_p$ OH, $O(CH_2)_p$ COOH, $O(CH_2)_pCONH_2$,

$O(CH_2)_pCONOH$, $(CH_2)_pSO_3H$, $O(CH_2)_pSO_3NH_2$, $O(CH_2)_pNO_2$, $O(CH_2)_pPO_3H_2$, CF_2COOH and p is 1 to 4

In this embodiment of the invention the phosphorus-containing radicals are phosphonates or phospates or derivatives thereof.

In one embodiment of the invention the backbone to the high density negatively charged region of vicinally oriented radicals is a cyclic moiety.

The cyclic moiety comprises a saturated or unsaturated aromatic or non-aromatic homo- or heterocyclic moiety. When the moiety is heterocyclic the heteroatoms are selected from the group of oxygen, nitrogen, sulfur or selenium.

Preferably the cyclic moiety comprises 4 to 24 atoms, most preferably 5 to 18 atoms. The cyclic moiety is for example selected from the group of cyclopentane, cyclohexane, cycloheptane, cyclooctane, inositol, monosacharide, disacharide, trisacharide, tetrasacharide, piperidin, tetrahydrothiopyran, 5-oxotetrahydrothiopyran, 5,5-dioxotetrahydrothiopyran, tetrahydroselenopyran, tetrahydrofuran, pyrrolidine, tetrahydrothiophene, 5-oxotetrahydrothiophene, 5,5-dioxotetrahydrothiophene, tetrahydroselenophene, benzene, cumene, mesitylene, naphtalene and phenantrene. When the cyclic moiety is an inositol it could be selected from the group of alloinositol, cisinositol, epiinositol, D/L-chiroinositol, scylloinositol, myoinositol, mucoinositol and neoinositol.

In one preferred embodiment of the intention the compounds are phosphates, phosphonates or phosphinates of cyclohexane such as 1, 2, 3-β -cyclohexane- 1,2,3-trioltrisphosphate.

In other preferred embodiments of this type of the invention the compounds are phosphates, phosphonates or phosphinates of inositol. Preferably the number of phosphate,

phosphonate or phosphinate radicals per inositol moiety is at least three. The remaining hydroxyl groups on the inositol moiety may be derivatized in the form of ethers or esters.

In one preferred embodiment the compound is myo-inositol-1,2,6-trisphosphate or myo-inositol- 1,2,3-trisphosphate.

In one most preferred embodiment the compounds are selected from the group of D-myo-inositol-1,2,6-trisphosphate, D-myo-inositol-1,2,6-tris(carboxymetylphosphate), D-myo-inositol-1,2,6-tris(carbomethylphosphonate), D-myo-inositol-1,2,6-tris(hydroxymethylphosphonate),D-3,4,5-tri-O-methyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-hexanoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-butanoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-pentanoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-isobutanoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-propanoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-(6-hydroxy-4-oxa)hexanoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-3-(ethylsulphonyl)propanoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-3-hydroxypropanoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-(6-hydroxy)-hexanoyl-myo-inositol-1,2,6-trisphosphate, D-5-O-hexanoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-phenylcarbamoyl-myo-inositol-1,2,6-trisphosphate, D-3,4,5-tri-O-propanoyl-myo-inositol-1,2,6-tris(carboxymethylphosphate), D-3,4,5-tri-O-butanoyl-myo-inositol-1,2,6-tris(carboxymethylphosphate), D-3,4,5-tri-O-isobutanoyl-myo-inositol-1,2,6-tris(carboxymethyl-phosphate), D-3,4,5-tri-O-pentanoyl-myo-inositol-1,2,6-tris(carboxymethylphosphate), D-3,4,5-tri-O-hexanoyl-myo-inositol-1,2,6-tris(carboxymethylphosphate), D-3,4,5-tri-O-propanoyl-myo-inositol-1,2,6-tris(carboxymethylpbosphonate), D-3,4,5-tri-O-butanoyl-myo-inositol-1,2,6-tris(carboxymethyl-phosphonate), D-3,4,5-tri-O-isobutanoyl-myo-inositol-1,2,6-tris(carboxymethylphosphonate), D-3,4,5-tri-O-pentanoyl-myo-

inositol-1,2,6-tris(carboxymethylphospbonate), D-3,4,5-tri-O-hexanoyl-myo-inositol-1,2,6-tris(carboxymethylphosphonate), D-3,4,5-tri-O-propanoyl-myo-inositol-1,2,6-tris(hydroxymethyl-phosphonate), D-3,4,5-tri-O-butanoyl-myo-inositol-1,2,6-tris(hydroxymethylphosphonate), D-3,4,5-tri-O-isobutanoyl-myo-inositol-1,2,6-tris(hydroxymethylphosphonate), D,-3,4,5-tri-O-pentanoyl-myo-inositol-1,2,6-tris(hydroxymethylphosphonate), D-3,4,5-tri-O-hexanoyl-myo-inositol-1,2,6-tris(hydroxymethyl-phosphonate).

When the cyclic moiety is a sacharide it could be selected from the group of D/L-ribose, D/L- arabinose, D/L-xylose, D/L-lyxose, D/L-allose, D/L-altrose, D/L- glucose, D/L-mannose, D/L- gulose, D/L-idose, D/L-galactose, D/L-talose, D/L- ribulose, D/L-xylulose, D/L-psicose, D/L-sorbose, D/L-tagatose and D/L-fructose or derivatives thereof. In preferred embodiments of this type of the invention the compounds are phosphates, phosphonates or phosphinates of sacharides. Preferably the number of phosphate, phosphonate or phosphinate radicals per sacharide unit is at least three. The remaining hydroxyl groups on the sacharide moiety may be derivatized in the form of ethers or esters. In many instances the ether form is desired as this type of radical prolongs the stability and half-life in vivo as the susceptibility to enzymatic degradation is reduced.

In one preferred embodiment of this type of the invention the compound is selected from the group of mannose-2,3,4-trisphosphate, galactose-2,3,4-trisphosphate, fructose-2,3,4-trisphosphate, altrose-2,3,4-trisphosphate and rhamnose-2,3,4-trisphosphate. In one most preferred embodiment the compound is selected from the group of R^1-6-O-R^2-α-D-manno-pyranoside-2,3,4-trisphosphate, R^1-6-O-R^2-α-D-galacto-pyranoside-2,3,4-trisphosphate, R^1-6-O-R^2-α-D-altropyranoside-2,3,4-trisphosphate and R^1-6-O-R^2-β-D-fructopyranoside-2,3,4-trisphosphate where R^1 and R^2 independently are as defined

above and preferably are methyl, ethyl, propyl, butyl, pentyl or hexyl. Most preferred compounds in this type of the invention are methyl-6-O-butyl-α-D-mannopyranoside-2,3,4-trisphosphate, methyl-6-O-butyl-α-D-galactopyranoside-2,3,4-trisphosphate, methyl-6-O-butyl-α-D-glycopyranoside-2,3,4-trisphosphate, methyl-6-O-butyl-α-D-altropyranoside-2,3,4-trisphosphate, methyl-6-O-butyl-β-D-fructopyranoside-2,3,4-trisphosphate, 1,5-anhydro-D-arabinitol-2,3,4-trisphosphate, 1,5-anhydroxylitol-2,3,4-trisphosphate, 1,2-O-ethylene-β-D-fructopyranoside-2,3,4-trisphosphate, methyl-α-D-rhamnopyranoside-2,3,4-trisphosphate, methyl-α-D-mannopyranoside-2,3,4-trisphosphate, methyl-6-O-butyl-α-D-mannopyranoside-2,3,4-tris-(carboxymethylphosphate), methyl-6-O-butyl-α-D mannopyranoside-2,3,4-tris(carboxymethylphosphonate), methyl-6-O-butyl-α-D-mannopyranoside-2,3,4-tris(hydroxymethylphosphonate), methyl-6-O-butyl-α-D-galactopyranoside-2,3,4-tris(carboxymethylphosphate), methyl-6-O-butyl-α-D-galactopyranoside-2,3,4-tris(carboxymethylphosphonate), methyl-6-O-butyl-α-D-galactopyranoside-2,3,4-tris(hydroxymethylphosphonate), methyl-6-O-butyl-α-D-glucopyranoside-2,3,4-tris(carboxymethylphosphate), methyl-6-O-butyl-α-D-glucopyranoside-2,3,4-tris(carboxymethylphosphonate), methyl-6-O-butyl-α-D-glucopyranoside-2,3,4-tris(hydroxymethylphosphonate), methyl-6-O-butyl-α-D-altropyranoside-2,3,4-tris-(carboxymethylphosphate), methyl-6-O-butyl-α-D-altropyranoside-2,3,4-tris-(carboxymethylphosphonate), methyl-6-O-butyl-α-D-altropyranoside-2,3,4-tris-(hydroxymethylphosphonate), methyl-6-O-butyl-β-D-fructopyranoside-2,3,4-tris-(carboxymethylphosphate), methyl-6-O-butyl-β-D-fructopyranoside-2,3,4-tris-

(carboxymethylphosphonate), methyl-6-O-butyl-β-D-fructo-pyranoside-2,3,4-tris-(hydroxymethylphosphonate).

In other preferred embodiments of the invention the compounds are phosphates, phosphonates or phosphinates of heterocyclic moieties such as 1,5-dideoxy-1,5-iminoarabinitol-2,3,4-trisphosphate,1,5-dideoxy-1,5-iminoarabinitol-2,3,4-tris-(carboxymethylphosphate), 1,5-dideoxy-1,5-imino-arabinitol-2,3,4-tris(carboxymethylphosphonate), 1,5-dideoxy-1,5-iminoarabinitol-2,3,4-tris(hydroxymethylphosphonate), 1,5-dideoxy-1,5-imino-N-(2-phenylethyl)arabinitol-2,3,4-trisphosphate, 1,5-dideoxy-1,5-imino-N-(2-phenylethyl)-arabinitol-2,3,4-tris(carboxymethylphosphate), 1,5-dideoxy-1,5-imino-N-(2-phenylethyl)arabinitol-2,3,4-tris-(carboxy-methylphosphonate), 1,5-dideoxy-1,5-imino-N-(2-phenyl-ethyl)arabinitol-2,3,4-tris(hydroxymethylphosphonate).

Within the process of restenosis, a number of regulatory substances are released. One category of substances are growth factors and the activity of these substances are considered to promote neointimal formation and hyperplasia. Ligand-induced dimerisation is a key event in transmembrane signalling by receptors with tyrosinase kinase activity. Unlike other growth factors such as platelet derived growth factor (PDGF) which are dimeric, fibroblast growth factors (FGF) are monomeric and are unable by themselves to induce activation of FGF receptors. Accordingly, FGF function in concert with for example heparin which induce dimerization and subsequent activation which leads to for example induction of transcriptional factors like early growth response factor 1 (Egr-1). It has been hypothesised that Egr 1 may play a key regulatory role by linking injurious stimuli to the induction of genes directing the expression of effector molecules in endothelial cells, smooth muscle cells, fibroblasts and leukocytes.

Such a complex between fibroblast growth factor 2 (FGF2) and its naturally occurring receptor 1 (FGFR1) has been structurally determined and it can be observed that a positively charged canyon formed by a cluster of basic amino acid residues represent the heparin-binding site. One type of activity of the described compounds according to the invention is to replace or take the place of heparin in this domain and thereby act as a blocking agent of fibroblast growth receptors. The consequence of this activity is to counteract the dimerization and subsequent activation, which leads to a down regulation of the detrimental effects of released substances and thereby a halt in the process of restenosis. Furthermore, another activity of the compounds according to the present invention is to affect the vascular remodelling which is an important factor in the process of restenosis. Remodelling is characterised by thickening and enlargement of existing cells and tissue. The geometry of vessels such as arteries can normally change in response to alterations in the local environment but the process of remodelling counteracts this compensatory response. In response to trauma, tissue such as adventitia, undergo a repair process in which fibroblasts are changed into myofibroblasts. This process is followed by deposition of extracellular matrix and results in geometric remodelling of the tissue. Up-regulation of the expression of the interstitial collagenase or matrix metalloproteinase gene and down-regulation of the synthesis of collagen are other events seen in the process of remodelling and restenosis. When administering the compounds according to the invention, one effect is that the process of remodelling is counteracted evidenced by a diminished transformation of fibroblasts into myofibroblasts, positive effects on collagen synthesis and a reduction of the deposition of extracellular matrix. Furthermore, an improved elasticity of the tissue and vessels are observed which shows a positive effect on the cytoskeletal

structure of the vessels. Hypertrophy of the heart occurs in response to cardiac wall stress induced by pressure or volume overload. The result is morphological, molecular and functional changes which is observed as increases in protein content, increase in cell size and so forth. These effects are counteracted by the use of the compounds according to the present invention.

The invention is also intended to be used for the preparing of a medicament for preventing, alleviating or combatting damages related to surgery and transplantation of organs and tissues. In these instances it is beneficial to administer the compounds before, during or after the surgery or transplantation. The effects of the compounds are also to effect wound contraction and to modify the scar formation in relation to for example surgery and transplantation in order to improve the surgical outcome. The administration of the compounds will for example be beneficial in connection to by-pass operations.

Furthermore, the administration of the compounds could be in connection to the placement of a stenting material, either by deposition of the compounds to the stenting material or by a local delivery to the injury.

According to the invention the compounds are most often present in a salt form or in a form where only a few of the negative charges are protonated. The salt can contain one or more cations in different combinations. Examples of cations are sodium and potassium ions.

The pharmaceutical composition according to the invention may be administered orally, topically, parentally, rectally or by inhalation spray in dosage forms or formulations comprising conventional, non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles.

The pharmaceutical composition for oral use can be present in different forms such as capsules, granules,

tablets, troches, lozenges, aqueous suspensions, dispensable powders, emulsions, syrups or elixirs. When the composition is present in liquid form capsules are preferably utilised. At the use of granules, these preferably have a size of 0,15-2 mm. The granules can either consist of the pharmaceutical composition per se or of the composition and suitable fillers. When the pharmaceutical composition is used in a tablet form, the tablets can have a weight of 50-1500 mg, preferably 50-800 mg and most preferably 100-500 mg.

Formulations for oral use include tablets, which contain the active ingredients in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium chloride, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, potato starch or alginic acid; binding agents, for example, starch, gelatin or acacia; and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay desintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin or olive oil.

For the parenteral application of the composition of this invention, typical dosage forms include intravenous, intramuscular and intraperitoneal formulations.

For the rectal application of the composition of this intention, typical dosage forms include suppositories, rectal

gelatin capsules (solutions and suspensions), and enemas or micro-enemas (solutions and suspensions). Thus, in a typical suppository formulation, any one of the compounds of this invention is combined with any pharmaceutically acceptable suppository base such as cocoa butter, esterified fatty acids, glycerinated gelatin and various water-soluble or dispersible bases like polyethylene glycols and polyoxyethylene sorbitan fatty acid ester. Various additives like salicylates or surfactant materials may be incorporated.

For topical use, creams, ointments, gels, solution or the alike containing the compositions are employed according to methods recognised in the art.

Naturally, the therapeutic dosage range for the compounds of the present intention will vary with the size and needs of the patient and the particular condition or disease symptom being treated.

The administration of the pharmaceutical composition according to the invention can be in a combined dosage form or in separate dosage forms.

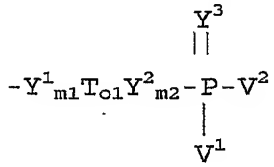
For administration to human patients appropriate dosages can routinely be determined by those skilled in this art by extension of the results obtained in animals at various dosages. The preferred dosage for humans falls within the range of 0,1 to 100 mg compound per day and kg body weight, especially 0,1 to 50 mg/day/kg body weight.

According to the present invention a process of preventing, alleviating or combatting restenosis by using a compound containing a high density, negatively charged domain of vicinally oriented radicals is described.

In preferred embodiments of the intention the negatively negatively charged domain comprises at least three vicinal phosphorus-containing radicals.

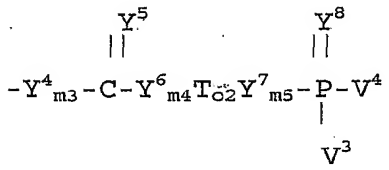
The invention also relates to a process wherein a compound containing phosphorus-containing radicals with the following formula is used as described above:

a)



or

b)



The invention will be further explained with the following embodiment examples however without limiting it thereto.

Example 1 and 2 describe the counteractive effects of D-myo-inositol-1,2,6-trisphosphate (IP_3) and D-3,4,5-tri-O-(phenylcarbamoyl) myo-inositol-1,2,6-trisphosphate (PP 11-201) on the proliferation of smooth muscle cells. Example 3 and 4 show the inhibitory effects of D-myo-inositol-1,2,6-trisphosphate (IP_3) and D-3,4,5-tri-O-(phenylcarbamoyl) myo-inositol-1,2,6-trisphosphate (PP 11- 201) on neointima formation. Example 5 discloses the interaction between the complex of Fibroblast Growth Factor 2 - Fibroblast Growth Factor Receptor 1 (FGF2 - FGFR1) and D-myo-inositol-1,2,6-trisphosphate.

Example 1

Vascular smooth muscle cell proliferation contributes to restenosis after coronary angioplasty. Human pulmonary artery smooth muscle cells (HPASMCs) were maintained in Medium

231 supplemented with Smooth muscle Growth Supplement. The cells, medium and supplement were obtained from Caascade Biologics Inc., Portland, OR, USA.

The cells were grown as monolayer culture in 100-mm diameter plastic tissue culture dishes (Nunc, Roskilde, Denmark) with 10 ml medium in 37 °C in a humidified atmosphere of 95% air and 5% CO_2 and subcultured at 4- to 6-day intervals. For measurement of cell growth, cells were seeded at density $5,0*10^3$/well in 96 well dishes in DMEM +0,2% FBS for 3 days. Culture medium was then removed and cells were seeded in DMEM + 0,2% FBS with 1 nM of basic fibroblast growth factor (FGF2). The addition of FGF2 induces cell proliferation of the HPASMCs. The FGF2 used was a recombinant human substance from Boehringer Mannheim Biochemica, Espoo, Finland. Various concentrations of D-myo-inositol-1,2,6-trisphosphate (IP_3) were added and the number of parallel wells in each treatment was four. The proliferation of cells was analysed after 24 hours of incubation by labelling each well with 0,2µCi of ^{3}H-thymidine for two hours. After that the cells were trypsinated and harvested on a filter. A Melt-on scintillator (Meltilex™ A; Wallac, Turku, Finland) was allowed to melt on the filter. The radioactivity of the sheets was counted on a liquid scintillator counter (1450 Microbeta Wallac, Turku, Finland).

Addition of FGF2 significantly increased the incorporation of ^{3}H-thymidine in DNA in HPASMCs, which is the measurement of the proliferation of the cells. Stimulation of proliferation was inhibited by the addition of IP_3 with the following result:

Substance	concentration(µM)	inhibition (%)
IP_3	1	61
	10	44
	100	68

The experiment shows that there is a strong effect of IP_3 to inhibit stimulated proliferation of smooth muscle cells.

Example 2

In a procedure similar to the one described in Example 1, D-3,4,5-tri-O-(phenylcarbamoyl) myo- inositol-1,2,6-trisphosphate (PP 11-201) was added with the result shown below:

Substance	concentration (μM)	inhibition (%)
PP 11-201	0,1	49
	1,0	61
	10	70

The experiment shows that there is a strong effect of PP 11-201 to inhibit stimulated proliferation of smooth muscle cells.

Example 3

The effects on neointima formation was assessed after inducing vessel injury in rats with a balloon catheter. Neointima formation is an important part in the process of restenosis. Adult mate Wistar rats (250-260 g) were housed under standard conditions and were fed with commercial rat chow and water ad libitum. During the experiment D-myo-inositol-1,2,6- trisphosphate (IP_3) was administered via subcutaneously operated osmotic minipumps with a dose level of 1 mg/kg/hr. A control group received saline solution by osmotic minipumps. The animals were anaesthetised with an intraperitoneal injection of ketamine, 100 mg/kg, (MTC Pharmaceuticals, Cambridge, Ontario, Canada) and xylazine, 10 mg/kg (Bayer Inc., Etobicoke, Ontario, Canada). Using a dissecting microscope, the left carotid artery was exposed on the ventral side of the neck via a midline incision. The bifurcation of the carotid artery was located and two ligatures were placed around the external carotid artery,

which was then tied off with the distal ligature. After temporarily occluding the internal carotid artery with a vascular clamp, a small incision was made between the two ligatures placed around the external carotid artery to introduce the endothelial denudation device, an inflated French Fogarty embolectomy catheter (Baxter Healthcare, Buckinghamshire, UK). Each animal was de-endothelialised by three passages of the catheter and the carotid artery was tied off proximal to the incision hole. The clamp was removed and the pulse of the carotid artery was rechecked. The skin incision was closed with a single suture. 14 days post-operatively, the animals were sedated. An abdominal incision was made to access the abdominal aorta for insertion of a cannula connected to a perfusion apparatus. The animals where killed by an overdose of anaestetic and then perfused with heparinised phosphate buffered saline (pH 7,4) at a rate of 100 ml/min per kg body weight and a pressure of 120 mm Hg. After replacement of saline, 4% paraformaldehyde in isotonic saline was introduced at the same flow rate. After fixation in situ, the carotids were dissected free. Three mid-carotid segments, approximately 10 mm long were rinsed and placed in 4% parafomaldehyde for another 16 hours before embedding and freezing in Tissue- Tek OCT media (Miles Inc., Elkhart, Indiana, USA). Frozen sections were stained with Movat's pentachrome and immunohistochemistry was performed using antibodies in order to examine the process of restenosis by measurement of the neointima formation. lt was observed that the administration of IP_3 reduced the formation as can be seen in the following table:

Substance	Neointima formation (mm^2)	Reduction (%)
Control	1,12	
IP_3	0,48	57

The experiment shows that the administration of IP_3 reduced neointima formation with 57 %, which describes a beneficial effect against restenosis. By microscopic investigation it could be observed that the elasticity of the vessel walls were improved in the animals receiving IP_3 compared to the vessel walls of the control group which shows a positive effect on remodelling.

Example 4

In a procedure similar to the one described in Example 3, D-3,4,5-tri-O-(phenylcarbamoyl) myo- inositol-1,2,6-trisphosphate (PP 11-201) was added with the result shown below:

Substance	Neointima formation (mm^2)	Reduction (%)
Control	1,12	
PP 11-201	0,43	62

The experiment shows that the administration of PP 11-201 reduced neointima formation with 62 %, which describes a beneficial effect against restenosis. By microscopic investigation it could be observed that the elasticity of the vessel walls were improved in the animals receiving PP 11-201 compared to the vessel walls of the control group which shows a positive effect on remodelling.

Example 5

The interaction between the complex of Fibroblast Growth Factor 2 - Fibroblast Growth Factor Receptor 1 (FGF2 - FGFR1) and a model compound, D-myo-inositol-1,2,6-trisphosphate, was studied with the Insight modelling package on Silicon Graphics platform using a manual 3-dimensional docking procedure. The protein X-ray structure of FGF2 - FGFR1

was according to Plotnikov et al, Cell 98, 641 (1999), Protein Data Bank entry 1CVS.

A long cleft of the protein complex (30 Å) exposes two binding sites to the model compound with the following characteristics:

Site 1.

Near the central part of the cleft there is a concentration of positively charged residues: Lys A26, Lys A135, Lys C163, Lys C166, Lys C172, Lys C175, Lys C207, Arg A120 and His C166. The following distances between the oxygen in the P-O radical in the model compound and the nitrogen atoms in the positively charged residues were characterized:

Residue	Distance (Å)
Lys C163	2,4
Lys A135	2,7
Lys C172	2,7
His C166	2,3

Site 2.

Near the end of the cleft region there is a concentration of another set of positively charged residues: Lys B26, Lys B135, Lys B120, Lys D172, Lys C175, Lys D163, Lys C207, Arg B120 and His D166. The following distances between the oxygen in the P-O radical in the model compound and the nitrogen atoms in the positively charged residues were characterized:

Residue	Distance (Å)
Lys B135	2,3
Lys D163	3,6
Lys C175	3,2
His D166	3,2

The modelling experiment shows a strong binding between the model compound, D-myo-inositol-1,2,6-trisphosphate, and two distinct sites of the FGF2 - FGFR1 receptor complex.

CLAIMS

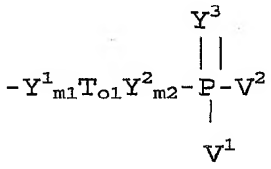
1. The use of a compound containing a high density negatively charged domain of vicinally oriented radicals for the preparing of a medicament for preventing, alleviating or combatting restenosis in mammals including man.

2. The use of a compound containing a high density, negatively charged domain of vicinally oriented radicals for the preparing of a medicament for preventing, alleviating or combatting hyperplasia, remodelling and hypertrophy in mammals including man.

3. The use according to anyone of claims 1-2 wherein the negatively charged domain comprises at least three vicinal phosphorus-containing radicals.

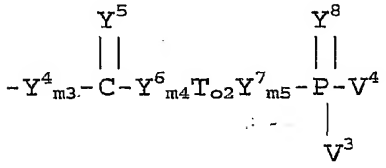
4. The use according to anyone of claims 1-2 wherein the phosphorus-containing radicals have the following formula:

a)



or

b)



wherein

V^1 to V^4 are $Y^8{}_{m6}T_{o3}U$

T_{o1} to T_{o3} are $(CH_2)_n$, CHCH, or $CH_2CHCHCH_2$

o1 to o3 are 0 to 1

n is 0 to 4

U is $R^1Y^9{}_{m7}$, $CY^{10}Y^{11}R^2$, $SY^{12}Y^{13}Y^{14}R^3$, $PY^{15}Y^{16}Y^{17}R^4R^5$, $Y^{18}PY^{19}Y^{20}Y^{21}R^6R^7$, CH_2NO_2, $NHSO_2R^8$ or $NHCY^{22}Y^{23}R^9$

m1 to m7 are 0 to 1

Y^1 to Y^{23} are N R^{10}, NOR^{11}, O or S

and where R^1 to R^{11} are

i) hydrogen

ii) a straight or branched saturated or unsaturated alkyl residue containing 1-22 carbon atoms

iii) a saturated or unsaturated aromatic or non-aromatic homo- or heterocyclic residue containing 3-22 carbon atoms and 0-5 heteroatoms consisting or nitrogen, oxygen or sulfur

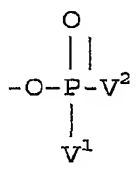
iv) a straight or branched saturated or unsaturated alkyI residue containing 1-22 carbon atoms substituted with a saturated or unsaturated aromatic or non-aromatic homo- or heterocyclic residue containing 3-22 carbon atoms and 0-5 heteroatoms consisting of nitrogen, oxygen or sulfur

v) an aromatic or non-aromatic homo- or heterocyclic residue containing 3-22 carbon atoms and O-5 heteroatoms consisting of nitrogen, oxygen or sulfur substituted with a straight or branched saturated or unsaturated alkyl residue containing 1-22 carbon atoms.

in the said groups ii-v, the residues and/or the substituents thereof being substituted with 0-6 of the following groups: hydroxy, alkoxy, aryloxy, acyloxy, carboxy, alkoxycarbonyl, alkoxycarbonyloxy, aryloxycarbonyl, aryloxycarbonyloxy, carbamoyl, fluoro, chloro, bromo, azido, cyano, oxo, oxa, amino, imino,

alkylamino, arylamino, acylamino, arylazo, nitro, alkylthio or alkylsulfonyl.

5. The use according to claim 4 wherein the phosphorus containing radicals have the following formula:



wherein V^1 and V^2 are OH, $(CH_2)_p$ OH, COOH, $CONH_2$, CONOH, $(CH_2)_pCOOH$, $(CH_2)_pCONH_2$, $(CH_2)_pCONOH$, $(CH_2)_pSO_3H$, $(CH_2)_pSO_3NH_2$, $(CH_2)_pNO_2$, $(CH_2)_pPO_3H_2$, $O(CH_2)_p$ OH, $O(CH_2)_p$ COOH, $O(CH_2)_pCONH_2$, $O(CH_2)_pCONOH$, $(CH_2)_pSO_3H$, $O(CH_2)_pSO_3NH_2$, $O(CH_2)_pNO_2$, $O(CH_2)_pPO_3H_2$, CF_2COOH
and p is 1 to 4

6. The use according to claim 4 wherein the phosphorus-containing radicals are phosphate groups.

7. The use according to anyone of claims 1-2 wherein a backbone to the high density negatively charged region of vicinally oriented radicals is a cyclic moiety.

8. The use according to claim 7 wherein the backbone is a saturated or unsaturated aromatic or non-aromatic homo- or heterocyclic moiety where the heteroatom is nitrogen, oxygen, sulfur or selenium.

9. The use according to claim 8 wherein the cyclic moiety comprises 4 to 24 atoms, preferably 5 to 18 atoms.

10. The use according to claim 8 wherein the cyclic moiety is selected from the group of cyclopentane, cyclohexane, cycloheptane, inositol, monosacharide, disacharide,

trisacharide, tetrasacharide, piperidin, tetrahydrothiophyran, 5-oxotetrahydrothiopyran, 5,5-dioxotetrahydrothiopyran, tetrahydroselenophyran, tetrahydrofuran, pyrrolidine, tetrahydrothiophene, 5-oxotetrahydrothiophene, 5,5-dioxotetrahydrothiophene, tetrahydroselenophene, benzene, cumene, mesitylene, naphtalene and phenanthrene.

11. The use according to claim 8 where in the cyclic moiety is selected from the group of alloinositol, cisinositol, epiinositol, D/L-chiroinositol, scylloinositol, myoinositol, mucoinositol and neoinositol.

12. The use according to claim 8 wherein the cyclic moiety is selected from the group of D/L-ribose, D/L-arabinose, D/L-xylose, D/L-lyxose, D/L-allose, D/L-altrose, D/L-glucose, D/L-mannose, D/L-gulose, D/L-idose, D/L-galactose, D/L-talose, D/L-ribulose, D/L-xylulose, D/L-psicose, D/L-sorbose, D/L-tagatose and D/L-fructose.

13. The use according to claim 8 wherein one of the phosphorus-containing radicals is axial and, two of the phosphorus-containing radicals are equatorial.

14. The use according to claim 13 wherein the compound is selected from the group of myo-inositol-1,2,6-trisphosphate, mannose-2,3,4-trisphosphate, rhamnose-2,3,4-trisphosphate, galactose-2,3,4-trisphosphate, methyl-6-O-butyl-α-D-mannopyranoside-2,3,4- trisphosphate, 1,5-anhydro-D-arabinitol-2,3,4-trisphosphate, fructose-2,3,4-trisphosphate, 1,2-O-ethylene-β-D-fructopyranoside-2,3,4-trisphosphate, cyclohexane-1,2,3-triol trisphosphate, 1,5-dideoxy-1,5-iminoarabinitol-2,3,4-trisphosphate, altrose-

2,3,4-trisphosphate, methyl-6-O-butyl-α-D-altropyranoside-2,3,4-trisphosphate or derivatives thereof.

15. The use according to anyone of claims 1-2 wherein the compound is administered by parenteral or non-parenteral administration.

16. The use according to anyone of claims 1-2 wherein the effective amount is from about 0,1 to about 100 mg per kg bodyweight of the animal or man.

17. The use according to anyone of claims 1-2 where in the medicament is in unit dosage form comprising tablets, granules, capsules, solutions or suspensions.

18. A process of preventing, alleviating, combatting restenosis by using a compound according to claims 1-17.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/01016

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 31/662
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9724111 A2 (HYAL PHARMACEUTICAL CORPORATION), 10 July 1997 (10.07.97) -- --------	

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 Sept 2002	27-09-2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Göran Karlsson/EÖ Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE02/01016

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 18
because they relate to subject matter not required to be searched by this Authority, namely:

A method for treatment of the human or animal body by therapy, see rule 39.1

2. ☒ Claims Nos.: 1-17
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

The compounds according to the claims are so vaguely defined and contain so many possible compounds which render it difficult, if not impossible to determine the matter for which protection is sought. The present application therefore fails to comply with the clarity and conciseness requirements of Article 6 PCT to such an extent that a meaningful search on the basis of the claims is impossible.

Due to these deficiencies, the search has been aimed at the compounds disclosed in the examples.

The applicants attention is drawn to the fact that claims relating to those parts of the inventions in which no international search report has been established will not be the subject of an international preliminary examination (Rule 66.1(e) PCT). This is the case irrespective of whether or not the claims are amended following receipt of the search report during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/09/02

International application No.
PCT/SE 02/01016

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9724111 A2	10/07/97	AU 725478 B	12/10/00
		AU 1091297 A	28/07/97
		BR 9612355 A	28/12/99
		CA 2166155 A	28/06/97
		EP 0874624 A	04/11/98
		HU 9902059 A	28/04/00
		IL 124828 D	00/00/00
		JP 2000506502 T	30/05/00
		NO 982935 A	27/08/98
		PL 327542 A	21/12/98
		ZA 9610840 A	27/06/97